

WHAT IS CLAIMED IS:

1                   1.       A method of determining the ability of a *Mycobacterium*  
2 *tuberculosis* bacterium to oxidize a thioamide or a thiocarbonyl, said method comprising  
3 detecting a mutation in an EtaA gene (SEQ ID NO:1) in said bacterium, wherein  
4 detection of the mutation is indicative of decreased ability to oxidize a thioamide or a  
5 thiocarbonyl.

1                   2.       The method of claim 1, wherein the mutation is a frameshift  
2 mutation selected from the group consisting of: a deletion at position 65, an addition at  
3 position 567, and an addition at position 811.

1                   3.       The method of claim 1, wherein the mutation is a single nucleotide  
2 polymorphism which causes an amino acid substitution in an amino acid sequence  
3 encoded by said EtaA gene compared to an amino acid sequence of SEQ ID NO:2.

1                   4.       The method of claim 3, wherein the single nucleotide  
2 polymorphism causes an amino acid substitution selected from the group consisting of:  
3 G43C, P51L, D58A, Y84D, T186K, T342K, and A381P.

1                   5.       A method of claim 1 wherein the mutation is detected by  
2                   (a) amplifying the EtaA gene, or a portion thereof containing the  
3 mutation, with a set of primers to provide an amplified product,  
4                   (b) sequencing the amplified product to obtain a sequence, and  
5                   (c) comparing the sequence of the amplified product with the  
6 sequence of a wild-type EtaA gene (SEQ ID NO:1) or portion thereof,  
7 wherein a difference between the sequence of the amplified product and the sequence of  
8 the wild-type EtaA gene or portion thereof indicates the presence of a mutation.

1                   6.       A method of claim 5, wherein at least one of said primers is  
2 selected from the group consisting of:  
3 5'-GGGGTACCGACATTACGTTGATAGCGTGGA-3' (SEQ ID NO:3),  
4 5'-ATAAGAATGCGGCCGCAACCGTCGCTAAAGCTAAACC-3' (SEQ ID NO:4),  
5 5' ATCATCCATCCGCAGCAC 3' (SEQ ID NO:5);  
6 5' AAGCTGCAGGTTCAACC 3' (SEQ ID NO:6);  
7 5' GCATCGTGACGTGCTTG 3' (SEQ ID NO:7);

8 5' AAGCTGCAGGTTCAACC 3' (SEQ ID NO:8);  
 9 5' TGAAGTCAGGTCGCGAAC 3' (SEQ ID NO:9);  
 10 5' AACATCGTCGTGATCGG 3' (SEQ ID NO:10);  
 11 5' ATTTGTTCCGTTATCCC 3' (SEQ ID NO:11);  
 12 5' AACCTAGCGTGTACATG 3' (SEQ ID NO:12);  
 13 5' TCTATTTCCCATCCAAG 3 (SEQ ID NO:13); and  
 14 5' GCCATGTCGGCTTGATTG 3' (SEQ ID NO:14).

1 7. A method of claim 5, wherein the primers are  
 2 5'-GGGGTACCGACATTACGTTGATAGCGTGGA-3' (SEQ ID NO:3), and  
 3 5'-ATAAGAATGCGGCCGCAACCGTCGCTAAAGCTAAACC-3' (SEQ ID NO:4).

1 8. A method of claim 5, wherein said amplification is by polymerase  
 2 chain reaction.

1 9. A method of claim 1, wherein said mutation is detected by  
 2 hybridizing DNA from said bacterium to a test nucleic acid under stringent conditions.

1 10. A method of claim 9, wherein either said DNA from said bacterium  
 2 or said test nucleic acid is immobilized on a solid support.

1 11. A method of claim 1, wherein said mutation is detected by  
 2 (a) subjecting said EtaA gene to digestion by restriction enzymes,  
 3 (b) separating the resulting restriction products to form a pattern of  
 4 restriction fragment lengths, and  
 5 (c) comparing the pattern of restriction fragment lengths to a  
 6 pattern of restriction fragment lengths formed by subjecting a known EtaA gene to the  
 7 same restriction enzymes.

1 12. A method of claim 11, wherein said known EtaA gene is selected  
 2 from the group consisting of (a) a frameshift mutation consisting of a deletion at position  
 3 65, an addition at position 567, and an addition at position 811, and (b) a single  
 4 nucleotide polymorphism which causes an amino acid substitution selected from the  
 5 group consisting of: G43C, P51L, D58A, Y84D, T186K, T342K, and A381P.

1                   13.     A method of claim 1, wherein said mutation is detected by  
2 specifically binding an antibody to a mutated product of the EtaA gene, wherein the  
3 specific binding of the antibody to the mutated gene product is indicative of a mutation  
4 which inhibits the ability of the bacterium to oxidize a thioamide.

1                   14.     A method of claim 13, wherein said gene product is in, or is  
2 isolated from, sputum.

1                   15.     A method of claim 13, wherein detection of said specific binding of  
2 said antibody and said mutated gene product is by ELISA.

1                   16.     A method of claim 1, wherein said thioamide or thiocarbonyl is  
2 selected from the group consisting of ethionamide, thiacetazone, and thiocarlide.

1                   17.     A method of claim 1, wherein said mutation is detected by  
2 (a) culturing said bacterium in the presence of ethionamide; and  
3 (b) testing for the presence or absence of (2-ethyl-pyridin-4-yl)methanol,  
4 wherein the absence of (2-ethyl-pyridin-4-yl)methanol indicates that the bacterium has a  
5 mutation which is indicative of decreased ability to oxidize a thioamide.

1                   18     A method of claim 17 wherein the presence or absence of (2-ethyl-  
2 pyridin-4-yl)methanol is tested by subjecting a medium in which the bacterium is  
3 cultured, or the bacterium, to analysis by thin-layer chromatography, high pressure liquid  
4 chromatography, or mass spectrometry.

1                   19     A method of claim 17, wherein the ethionamide of step (a) is  
2 radioactively labeled.

1                   20.     A method of claim 17, wherein the (2-ethyl-pyridin-4-yl)methanol  
2 is radioactively labeled.

1                   21.     A method of screening an individual for a *Mycobacterium*  
2 *tuberculosis* bacterium resistant to treatment by a thioamide or a thiocarbonyl drug,  
3 comprising  
4 (a) obtaining a biological sample containing said bacterium from said  
5 individual, and

6 (b) detecting a mutation in an EtaA gene (SEQ ID NO:1) in said  
7 bacterium, wherein detection of the mutation is indicative said bacterium is resistant to  
8 treatment by a thioamide or a thiocarbonyl drug.

1 22. A method of claim 21, wherein the mutation is detected by  
2 (a) amplifying the EtaA gene with a set of primers to provide an  
3 amplified product,  
4 (b) sequencing the amplified product to obtain a sequence, and  
5 (c) comparing the sequence of the amplified product with the  
6 sequence of a wild-type EtaA gene (SEQ ID NO:1),  
7 wherein a difference between the sequence of the amplified product and  
8 the sequence of the wild-type EtaA gene indicates the presence of a mutation.

1 23. A method of claim 21, wherein at least one of said primers is  
2 selected from the group consisting of:  
3 5'-GGGGTACCGACATTACGTTGATAGCGTGGA-3' (SEQ ID NO:3),  
4 5'-ATAAGAATGCGGCCGCAACCGTCGCTAAAGCTAAACC-3' (SEQ ID NO:4), 5'  
5 ATCATCCATCCGCAGCAC 3' (SEQ ID NO:5);  
6 5' AAGCTGCAGGTTCAACC 3' (SEQ ID NO:6);  
7 5' GCATCGTGACGTGCTTG 3' (SEQ ID NO:7);  
8 5' AAGCTGCAGGTTCAACC 3' (SEQ ID NO:8);  
9 5' TGAAGTCAGGTCGCGAAC 3' (SEQ ID NO:9);  
10 5' AACATCGTCGTGATCGG 3' (SEQ ID NO:10);  
11 5' ATTTGTTCCGTTATCCC 3' (SEQ ID NO:11);  
12 5' AACCTAGCGTGATACATG 3' (SEQ ID NO:12);  
13 5' TCTATTTCCCATCCAAG 3 (SEQ ID NO:13); and  
14 5' GCCATGTCGGCTTGATTG 3' (SEQ ID NO:14).

1 24. A method of claim 21, wherein said primers are  
2 5'-GGGGTACCGACATTACGTTGATAGCGTGGA-3' (SEQ ID NO:3) and 5'-  
3 ATAAGAATGCGGCCGCAACCGTCGCTAAAGCTAAACC-3' (SEQ ID NO:4).

1 25. A kit for determining the ability of a *Mycobacterium tuberculosis*  
2 bacterium to oxidize a thioamide or a thiocarbonyl, the kit comprising:  
3 (a) a container, and

4 (b) primers for amplifying an EtaA gene of said bacterium or a portion of  
5 said EtaA gene containing a mutation affecting the ability of the bacterium to oxidize a  
6 thioamide.

1 26. A kit of claim 25, wherein at least one of said primers is selected  
2 from the group consisting of:  
3 5'-GGGGTACCGACATTACGTTGATAGCGTGGA-3' (SEQ ID NO:3),  
4 5'-ATAAGAATGCGGCCGCAACCGTCGCTAAAGCTAAACC-3' (SEQ ID NO:4),  
5 5' ATCATCCATCCGCAGCAC 3' (SEQ ID NO:5);  
6 5' AAGCTGCAGGTTCAACC 3' (SEQ ID NO:6);  
7 5' GCATCGTGACGTGCTTG 3' (SEQ ID NO:7);  
8 5' AAGCTGCAGGTTCAACC 3' (SEQ ID NO:8);  
9 5' TGAACTCAGGTCGCGAAC 3' (SEQ ID NO:9);  
10 5' AACATCGTCGTGATCGG 3' (SEQ ID NO:10);  
11 5' ATTTGTTCCGTTATCCC 3' (SEQ ID NO:11);  
12 5' AACCTAGCGTGACATG 3' (SEQ ID NO:12);  
13 5' TCTATTTCCCATCCAAG 3 (SEQ ID NO:13); and  
14 5' GCCATGTCGGCTTGATTG 3' (SEQ ID NO:14).

1 27. A kit of claim 25, wherein the primers are  
2 5'-GGGGTACCGACATTACGTTGATAGCGTGGA-3' (SEQ ID NO:3), and  
3 5'-ATAAGAATGCGGCCGCAACCGTCGCTAAAGCTAAACC-3' (SEQ ID NO:4).

1 28. A kit of claim 25, further comprising a mutated EtaA gene for use  
2 as a positive control.

1 29. A kit of claim 28, wherein said mutated EtaA gene is selected from  
2 the group consisting of (a) a frameshift mutation consisting of a deletion at position 65, an  
3 addition at position 567, and an addition at position 811, and (b) a single nucleotide  
4 polymorphism which causes an amino acid substitution selected from the group  
5 consisting of: G43C, P51L, D58A, Y84D, T186K, T342K, and A381P.

1 30. A kit for determining the ability of a *Mycobacterium tuberculosis*  
2 bacterium to oxidize a thioamide, the kit comprising:

- 3 (a) a container, and  
4 (b) (2-ethyl-pyridin-4-yl)methanol.

1                    31.     A kit for determining the ability of a *Mycobacterium tuberculosis*  
2 bacterium to oxidize a thioamide, the kit comprising:

- 3                    (a) a container, and  
4                    (b) radiolabeled ethioamide.

1                    32.     A kit for determining the ability of a *Mycobacterium tuberculosis*  
2 bacterium to oxidize a thioamide or thiocarbonyl, the kit comprising:

- 3                    (a) a container, and  
4                    (b) an antibody which specifically binds to a product of a EtaA gene  
5 selected from the group consisting of a wild-type EtaA gene (SEQ ID NO:1) and a  
6 mutated EtaA gene.

1                    33.     A kit for determining the ability of a *Mycobacterium tuberculosis*  
2 bacterium to oxidize a thioamide, the kit comprising:

- 3                    (a) a container, and  
4                    (b) an antibody which specifically binds to (2-ethyl-pyridin-4-  
5 yl)methanol.